significant and can affect results where proteins dissolved in these detergents are used. The mechanisms behind these phenomena have yet to be elucidated; however, it is likely that these detergents modify membrane elasticity in different ways, resulting in either partial solubilization of the ion channel or enhanced rigidity of the membrane surrounding the channel.

## Inward Rectifier K Channels

**2729-Pos Board B499**

**Crystal Structure of a Prokaryotic Kir Channel in an Open Conformation**

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KirBac channels are prokaryotic homologs of mammalian inwardly-rectifying (Kir) potassium channels and recent X-ray crystal structures of both Kir and KirBac channels have provided a major insight into their unique structural architecture. However, all of the available structures are closed at the helix bundle-crossing and therefore the structural mechanisms which control opening of their primary activation gate remain unknown. Previous studies suggest that the open state of the Kir channel state is energetically unstable and will thus be difficult to trap in a crystal form. To circumvent this problem we have engineered KirBac3.1 to trap the bundle-crossing in an apparently open conformation, and we present the crystal structure of this mutant channel at 3.05 Å resolution. Contrary to previous speculation, this novel structure now suggests a mechanistic model in which rotational 'twist' of the intracellular assembly is converted into opening of the bundle-crossing gate via a network of inter- and intra-subunit interactions that couples the TM2 C-linker to the slide-helix, the G-loop and the CD-loop.

**2730-Pos Board B500**

**Comparison of the Structure of a Bacterial Potassium Channel in Both 2D and 3D Crystals**

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Inwardly-rectifying potassium (Kir) channels regulate membrane electrical excitability and K+ transport in many cell types where they control such diverse processes as heart rate, vascular tone, insulin secretion and salt/fluid balance. Their physiological importance is highlighted by the fact that genetically inherited defects in Kir channels are responsible for a wide-range of channelopathies. To elucidate how channel function becomes defective in the disease state requires a detailed understanding of channel structure in both the open and closed states, but to date detailed information about the open state structure of the Kir channel is lacking. In this work, we have used EM analysis of 2D crystals of a prokaryotic Kir channel trapped in an open state and compared these results with an open state structure of the same channel that our lab recently determined by X-ray crystallography at 3Å resolution. Intriguingly, the projection maps from the EM experiments suggest a larger opening of the pore in the 2D crystal form compared to that observed in the 3D crystal structure. The organization of these two crystal forms is different and suggests that the 2D crystals may permit stabilisation of an open state structure that is not compatible with 3D crystallisation. These results not only have major implications for our understanding of the open state structure of the Kir channel, but more importantly they demonstrate the general utility and importance of methods such as electron microscopy and 2D crystallography for the study of membrane protein structure.

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**2731-Pos Board B501**

**Molecular Basis of $Pip_2$ Gating KirBac1.1**

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KirBac1.1 is a bacterial inward rectifier potassium (Kir) channel, which, in contrast to its eukaryotic homologues, is strongly inhibited by phosphatidylinositol-4,5-bisphosphate ($PIP_2$). Previously, we labeled introduced cysteines in the KirBac1.1 cytoplasmic domain with small molecule fluorophores to examine $PIP_2$ dependent structural rearrangements by FRET. The major cytoplasmic β-sheet tilts inwardly, and the small cytoplasmic β-sheet moves inwardly to narrow the channel pore upon $PIP_2$ inhibition. By scanning mutagenesis, we have identified several residues that are essential for $PIP_2$ inhibition of KirBac1.1, but away from the $PIP_2$ binding pocket. R165C and R165A maintain similar channel activity to that of wild type, whereas channel activities of N168C and R170C are significantly reduced. However, all four mutations abolish $PIP_2$ inhibition. On the R165A background, we introduced single cysteine throughout the KirBac1.1 cytoplasmic domain and studied $PIP_2$-induced structural rearrangements using small molecule fluorophore FRET. Compared with the wild type background, the R165A mutation also abolishes the $PIP_2$ induced tilting motion of the major cytoplasmic β-sheet, and the inward movements of the small cytoplasmic β-sheet parallel to the pore axis. Thus R165A uncouples $PIP_2$ binding from channel closure, as well as the associated movements of the cytoplasmic domain, confirming that the wild type cytoplasmic domain motions are necessary for channel closure.

**2732-Pos Board B502**

**The Molecular Basis of Phosphoinositide Activation of Human Inward Rectifier (Kir2.1) Channels**

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Inward rectifier potassium (Kir) channels are directly regulated by phospholipids, including phosphoinositides (PIPs) and anionic glycerophospholipids (ALs) in the absence of other proteins or downstream signaling pathways. In the presence of non-specific bulk ALs Kir2.1 channels are highly selective for activation by $PI(4,5)P_2$, and under certain lipid conditions are inhibited by other PIPs. Biochemical approaches with purified full-length Kir2.1 channels enabled us to identify specific residues that regulate binding for 6 of 7 PIP ligands, and to estimate the energetic contributions of each of these residues to the binding of each PIP. A recent $PI(4,5)P_2$ bound Kir2.2 crystal structure (PDB entry 3SPI) suggests that $PI(4,5)P_2$ interacts directly with residues R80, R82, R182, K185, K187, K189 in Kir2.1. While K185 contributes ~0.89 kcal/mol to the binding of $PI(4,5)P_2$, the other residues in this binding pocket did not alter the affinity (and thus binding energy) of ligand binding. However, when R189, R218 and R219 residues, which do not reside in this binding pocket, were mutated to glutamine, the binding affinity for $PI(4,5)P_2$ was markedly reduced compared to WT, with calculated ΔΔG's of ~0.44, ~1.24 and ~0.85 kcal/mol, respectively. Notably, the particular subsets of residues that when mutated disrupt binding are different for each PIP. We further employed ligand docking approaches on homology models of human Kir2.1 channels based on chicken Kir2.2 crystal structures to identify putative binding regions for these PIPs and ALs. These simulations suggest that different PIPs may bind in similar but non-identical locations, in multiple poses. Our combined biochemical and computational analyses provide insight to the complexities of variable specificity, competition and synergy between different phospholipids in regulation of Kir channel gating.

**2733-Pos Board B503**

**Novel Chemical Probes for Polyamine Binding in the Inward-Rectifier Kir2.1**

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Inwardly rectifying potassium ($K_{ir}$) channels exhibit preferential conduction of currents into the cell, due to voltage-dependent block by intracellular polyamines. This function enables $K_{ir}$ channels to conduct near the resting membrane potential, but rapidly shut down to allow action potentials to proceed. We set out to explore the detailed chemical features of polyamine interactions within the pore of the prototypical inward rectifier $K_{ir}2.1$. Polyamines first interact with residues F254 and D255 in the channel's cytoplasmic domain to produce low-affinity block. Alteration of residue F254 by introduction of non-natural fluorinated phenylalanine analogs, using stop codon suppression, did not reduce low-affinity spermine block, ruling out a hypothesized cation-π interaction with spermine in the cytoplasmic domain. To examine the high-affinity inner cavity binding site, we characterized the blocking kinetics and affinity of a family of novel synthetic polyamine analogs, with progressive alkylation of terminal amines. Increasing blocker length or terminal amine volume decelerated blocking/unblocking kinetics, but did not change the voltage-dependence of block. These results imply that access to the high-affinity site is sterically hindered, but blocker entry to the constrained selectivity filter does not contribute significantly to the steep voltage-dependence of block. Lastly, a novel hydrogen bond-deficient spermine analog showed greatly decreased affinity relative to spermine in $K_{ir}2.1$, but not in $K_{ir}2.1$[D172N], illustrating the importance of close amine-carboxylate interactions to high-affinity block. Similarly, amine introduction at a substituted inner cavity cysteine (I176C) demonstrated that hydrogen bond-'enabled' modifiers (ie. MTSEA) disrupt spermine block more than hydrogen bond-deficient modifiers (ie. MTSET). These findings dissect the chemical details of multiple polyamine binding sites in Kir2.1, and demonstrate the critical contribution of hydrogen bonding to high-affinity spermine block.